Exmos. Srs.

Equipa Editorial Acta Médica Portuguesa

Gostaríamos de agradecer aos revisores as sugestões e comentários, que contribuíram para melhorar a qualidade do manuscrito. Após análise detalhada dos comentários, questões e sugestões, procedemos às alterações.

Enviamos o manuscrito 15122-60237-1, corrigido e revisto.

**Notas do editor:**

- O resumo e o abstract deverão reflectir fielmente a estrutura do artigo, pelo que é necessário que incluam um parágrafo independente relativo à secção "Conclusão".

*A estrutura de abstract foi modificada conforme indicado:*

*“Discussion: Estimated seroprevalence of SARS-COV-2 was higher than cumulative incidence reported by the National Surveillance System but far from necessary to reach herd immunity.*

*Conclusions: Our results support limited extent of infection by SARS-CoV-2 in the study population possibly due to early lockdown measures implemented in Portugal and support the need to continue monitoring of SARS-CoV-2 seroprevalence to increase our knowledge about epidemic´s evolution and to estimate the proportion of the susceptible population along time.”*

*As mesmas alterações foram feitas no resumo em Português.*

- A Figura 1 deverá ser carregada como novo documento suplementar, em formato vectorial, para que tenha qualidade para publicação.

*Foi preparado um ficheiro com a Figura 1 em formato vectorial*

**Revisor C:**

-Esta é uma contribuição muito relevante e original para o conhecimento da resposta imunológica da população portuguesa à primeira fase da pandemia por COVID-19 em Portugal, pelo não tenho qualquer dúvida de que deve ser publica na Acta Médica Portuguesa, nos primeiros 10% da prioridade de publicação.

Não tenho nenhum comentário significativo quanto aos objetivos, metodologia e apresentação do trabalho. Julgo, no entanto, que na conclusão do trabalho deveria haver uma menção relativa aos níveis de seropositividade encontrados, para além da expressão da necessidade em dar continuidade à sero-epidemiologia da COVID-19.

*A conclusão do trabalho foi reformulada conforme proposto:*

*“In May-July 2020 the observed prevalence of anti-SARS-CoV-2 specific antibodies was low in a survey population and comparable to other studies developed in similar settings with low incidence rates of COVID-19. Given the low sensitivity of the National Surveillance System to detect asymptomatic cases we emphasize the need of monitoring the distribution of specific antibodies against SARS-CoV-2 during the forthcoming months, especially after the second pandemic wave, aiming to estimate the fraction of SARS-CoV-2 susceptible population and supporting vaccination strategy. ”*

**Revisor D:**

-O presente trabalho apresenta a “fragilidade” de ter sido realizado com base numa amostragem aleatória (oportunista). Contudo, tem o mérito de ser um dos poucos estudos nacionais sobre seroprevalência da infeção por SARS-CoV-2.O texto apresenta pequenas gralhas de escrita (texto/abreviaturas) que necessitam ser revistas.

*As gralhas e abreviaturas foram corrigidas*

**Revisor E:**

- Agradecimentos: identifica a fonte de financiamento? Identifica conflitos de interesse? Referem não haver conflitos mas não identificam fontes de financiamento

*Foi adicionada uma descrição da fonte de financiamento*

Extensão: o manuscrito pode ser encurtado sem eliminar aspetos fundamentais? As figuras/tabelas podem ser eliminadas ou melhoradas? A extensão do manuscrito é adequada. A figura 1 não é suficientemente informativa (neste formato poderá ser substituída por uma tabela mais informativa, por exemplo com informação sobre o contributo de cada instituição incluída do estudo e caraterização da instituição (além da localização)

*Agradecemos a sugestão e para melhor informar os leitores juntamente com o mapa que ilustra a distribuição geográfica dos locais da colheita, adicionamos no texto o número de elementos recrutados nos laboratórios e em contexto hospitalar. “Overall 2,301 participants were recruited, 1.467 by clinical pathology laboratories network and 834 by public hospitals.”*

*A listagem de 96 laboratórios, 18 hospitais e 101 concelhos é demasiado extensa para ser incluída no corpo do artigo em formato de um quadro, pelo que optámos por não incluir um quadro com o contributo de cada posto de colheita em forma de tabela, como sugerido pelo Revisor.*

**Comentários específicos**

- No resumo descrever o processo de amostragem para que seja possível compreender a validade externa dos resultados.

*O abstract foi repormulado conforme sugerido, uma breve descrição de amostragem foi incluída nos métodos: “Cross-sectional seroepidemiological study was developed after the peak of the first epidemic wave using a non-random sample of 2,301 Portuguese residents, aged 1 year or older”.*

*O resumo em Portugues também foi reformulado*

- A conclusão deve ser reescrita para clarificar que não é uma amostra representativa da população e por isso não corresponde à prevalência na população Portuguesa.

*A conclusão de trabalho foi reformulada à luz do comentário: “Our results support limited extent of infection by SARS-CoV-2* *in the study population possibly due to early lockdown measures implemented in Portugal support the need to continue monitoring of SARS-CoV-2 seroprevalence to increase our knowledge about epidemic´s evolution and to estimate the proportion of the susceptible population along time”. Consideramos que com a adição do método de amostragem no abstract a questão de representatividade já se torna mais clara.*

**Introduction**

Clarificar a relevância e utilidade de conhecer a seroprevalência relativamente ao SARS-CoV-2 e o potencial contributo de um estudo com representatividade para diferentes caraterísticas pessoais e geográficas.

*Em resposta ao comentário foi adicionado na introdução o seguinte paragrafo:*

*“Given the limited knowledge of the epidemiological and serological characteristics of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the primary focus of national surveillance systems on patients with severe disease, the World Health Organization (WHO) recommended that countries should perform seroepidemiological surveys to investigate the extend of SARS-CoV-2 infection as determined by seropositivity in the general population2.*

*A standardized protocol for population-based age-stratified seroepidemiological investigations for SARS-COV-2 was proposed to better understand a spread of infection, including mild and subclinical cases that did not require medical care, and to allow comparability of results across countries”*

**Methods**

- Clarificar o processo de amostragem. Os autores referem: “Survey participants were recruited among those who visited partner laboratories or hospitals during the study period, for reasons unrelated to COVID-19 and with a medical prescription for a blood test that involved venipuncture” e “A non-probability sampling design (quota sampling) was used.”Não é claro os critérios para seleção dos laboratórios e hospitais incluídos, também não são descritos os critérios de inclusão e exclusão dos participantes (exceto a presença de patologia para a qual é necessário clarificar se é por prevalência ao longo da vida ou por doença ativa) nem a metodologia de seleção dos participantes a convidar. Não é descrita a proporção de participação entre os convidados para o estudo e as diferenças entre participantes e não participantes. Clarificar os procedimentos para amostragem dentro no mesmo cluster, por exemplo num conjunto de coabitantes.

*Em resposta ao comentário foi incluída no artigo uma descrição mais técnica e detalhada do processo de amostragem:*

*“A two-stage non-probability sampling design (quota sampling) stratified by age group was used. In order to estimate an expected seroprevalence of 2.5% in the 0-19 yo and of 5% in the 20 yo and over, with an absolute precision of 2% for 95% confidence interval and a design effect of 1.5, the required sample size was determined to be 2,072 individuals.*

*Sampling units, individuals, were allocated homogeneously by Health Region (North, Centre, Lisbon and Tagus Valley, Alentejo, Algarve, and the AR of Madeira and Azores) in order to obtain estimates with similar precision in all regions. To assure survey geographical coverage in North, Centre, Lisbon and Tagus Valley, and Alentejo regions sample size was allocated, proportionally to the distribution of resident population, by NUT III, while in Algarve, Autonomous Region (AR) Madeira and AR Azores by smaller geographical units, since each of these three regions consist of a single NUT III.*

*Within each NUT III or equivalent smaller geographical unit, municipalities were selected by simple random sampling. The number of municipalities to select in each NUT III was proportional to geographical unit population size within Health Region (in larger NUT III more municipalities were selected). The final number of municipalities selected was 101.  Within each of randomly selected municipality, data collection point were chosen, by convenience, from a nationwide network of private and public pathology laboratories. At least one data collection point was selected in each regional unit (NUT III level). The number of data collection points was determined taking into consideration logistical issues, such as the number of days for observation and capacity to recruit participants (17-22 individuals in each data collection point) after lockdown restrictions were lifted. The selected laboratories consisted of 96 clinical pathology laboratories and 18 public hospitals located in 101 randomly selected municipalities (Fig 1). Survey participants were recruited according pre-defined sex–age group quota distributed by Health Region (homogenously), by NUT III (proportional to population size), by selected municipality (homogeneously within NUT III or equivalent).”.*

*Os critérios de exclusão dos participantes, por patologia que afeta resposta imunitária, foram clarificados conforme solicitado:*

*“…, such as chronic liver disease, cancer in previous 5 years, immunodeficiency, organ transplantation or transfusion in previous 6 months were excluded.”*

*Relativamente à seleção dos participante, gostaríamos de clarificar que o método não probabilístico de seleção por quotas significa que em cada posto de colheita (cluster), os participantes foram recrutados por conveniência até completar a quota estabelecida. Os elementos da equipa de campo receberam a indicação de convidar para o estudo todos os indivíduos que que se desloquem ao posto para realizar análises por prescrição médica por motivos não relacionados com COVID-19, até completar a respetiva quota por sexo e grupo etário. Para todos os convidados era necessário verificar os critérios de elegibilidade e registar o resultado dessa verificação. No processo de verificação de elegibilidade foram excluídas 80 pessoas convidadas. Dado que o método de seleção não foi aleatório, na ausência de uma listagem previa das pessoas a convidar, não foram registadas as recusas, nem aplicado nenhum inquérito de não participação que permitisse comparar as características dos participantes e não participantes, também não foi calculada a taxa de resposta. Por estes motivos não é possível incluir no manuscrito um fluxograma de recrutamento e comparação dos participantes e não participantes.*

- Clarificar se o consentimento foi escrito e acrescentar que para os menores de idade o consentimento foi obtido pelos seus representantes/responsáveis legais.

*A indicação que o consentimento informado foi escrito e que foi assinado por participante ou por seu representante legal já se encontrava na versão submetida artigo. Para clarificar que “or their legal representatives” refere-se apenas aos menores de idade, a frase foi modificada de seguinte modo: All study participants or their legal representatives (for minors) signed written informed consent.*

- No cálculo do tamanho amostral não foi tido em conta o efeito de clustering que pode ser observado pela localização das instituições incluídas.

*Na descrição do cálculo do tamanho amostral foram incluídos 3 parâmetros: a prevalência esperada, a margem de erro e o* ***“design effect”****. O último corresponde o efeito conjunto de clustering e estratificação. Ambos foram tidos em conta nos cálculos: “The required sample size was determined to be of 2,072 individuals, considering an expected seroprevalence of 2.5% in the 0-19 yo and of 5% in the 20 yo and over, an absolute precision of 2% for 95% confidence interval and a* ***design effect of 1.5****.”*

- No subcapítulo Laboratory analysis sugiro acrescentar a informação sobre a sensibilidade e especificidade dos testes utilizados para facilitar a compreensão dos resultados observados.

*Conforme sugerido, foi adicionada a informação relativa à performance dos testes serológicos segundo as especificações do fabricante. “According to manufacture’s information, assay sensitivity was 86.1% and specificity was 99.4%. IgG against SARS-CoV-2 was determined by ELISA using the EUROIMMUN Anti-SARS-CoV-2 Assay on a fully automated I-2P Analyser. According to manufacture’s information, assay sensitivity was 94.4% from day 10 after symptoms onset while specificity was 99.6%.”*

*-* No item Case definitions a descrição dos “a) symptomatic” é confusa porque não se percebe o papel da anosmia uma vez que refere “symptomatic if they presented anosmia and/or three or more of the following symptoms”. Era a anosmia um critério necessário? Se não, é suficiente referir “three or more of the following symptoms…”

*Relativamente a definição apresentada, podemos clarificar que os indivíduos apenas com anosmia foram classificados como sintomáticos, bem como indivíduos que apresentavam 3 o mais sintomas entre os descritos. Na revisão do inglês foi nos aconselhado utilizar “and/or” para esta definição.*

-Seria importante apresentar a informação em função do diagnóstico anterior de COVID-19. Foi recolhida essa informação?

*Essa informação foi recolhida mas dado o baixo número de recrutados com teste positivo para SARS-CoV-2 não permite apresentar os resultados estratificados por esse fator, devido as limitações de foro estatístico.*

**Statistical analysis**

Descrever a informação utilizada para fazer a ponderação dos resultados.

*Foi adicionada no manuscrito uma referência ao documento e breve descrição do cálculo dos ponderadores. Para efetuar o cálculo foram utilizadas apenas as estimativas da população residente em 2019, disponíveis no site do Instituto Nacional de Estatística. Para corrigir a estrutura da amostra para o desenho amostral, a cada indivíduo pertencente ao grupo etário , sexo e região , foi atribuído um peso () dado por:*

*onde é a dimensão total da população, a dimensão total da amostra, a dimensão da amostra no grupo etário sexo e região e a dimensão da população no grupo etário , sexo e região .*

**Discussion**

A discussão deve ser reforçada, particularmente focando dois pontos:

-Discutir as possíveis explicações para não ter encontrado diferenças por idade e região (“No statistically significant differences in seroprevalence were observed by age group (p = 0.98) or region (p = 0.71) uma vez que a dinâmica da pandemia foi diferente por região. Será falta de poder para encontrar as diferenças?

*Gostaríamos de clarificar que o principal objetivo do estudo, para o qual a amostra foi dimensionada era estimar uma seroprevalência e não identificar diferenças entre diferentes grupos, pois esperávamos diferenças de reduzida magnitude, como pouco impacto em termo de saúde pública, mas que obrigariam ao recrutamento de um muito elevado número de participantes. Para ter potencia de 80% para detetar uma diferença entre 2.2% e 3.2% será necessária uma amostra de 4481 indivíduos em cada grupo de comparação, o que equivalente a 31367 para os 5 grupos etários considerados. De ponto de vista de saúde publica e de planeamento das intervenções, diferenças com magnitude na ordem de 1% não parecem ter grande relevância para a tomada de decisão. Deste modo, não nos pareceu relevante apresentar uma discussão profunda da ausência de diferenças nas taxas de prevalência por grupo etário e região.*

-Discutir as limitações do processo de amostragem para a validade externa dos resultados uma vez que a população que realiza colheitas de sangue para análises sanguíneas, particularmente em alguns grupos etários – os mais jovens – não representam a população geral. Este efeito poderá ser ainda mais relevantes no período em estudo (“The fieldwork took place between May 21 and July 8, 2020.”).

*Em resposta ao comentário foi adicionada no manuscrito uma discussão mais detalhada dos possíveis viés.*

*“Among limitations that could have affected the study results, we should mention the lack of representative sample of general Portuguese population because research has shown that surveys on SARS-CoV-2 seroprevalence may be seriously affected by self-selection or volunteer bias17 as individuals with previous diagnosis of COVID-19 volunteered more frequently to participat 17. To reduce this self-selection bias in the ISNCOVID-19 survey participants were recruited among those who already were in a laboratory with the objective to perform routine blood tests prescribed by a medical doctor for reasons unrelated to COVID-19. This methodological option, on the other hand, may have resulted in sampling of individuals with higher proportion of chronic conditions than in general population18, particularly among children, and this could have contributed to a lower seroprevalence resulting from a higher awareness and adoption of protection measures by these groups at risk. In addition, selecting participants among users of clinical pathology laboratories may have resulted in bias due to the documented educational gradient in access to complementary diagnostic tests18. However, the Portuguese Serological Survey for Vaccine Preventable Disease developed in 2015-2016 using similar sampling strategy among clinical pathologies laboratories and public hospital users did not demonstrate bias in the educational distribution of participants5. Exclusion of institutionalized individuals from the present survey may have contributed to underestimation of SARS-CoV-2 seroprevalence since, according to the national surveillance system, several institutional facilities for the elderly were affected by COVID-19 during the first wave.”.*

**Conclusion**

A conclusão apresentada não corresponde à resposta ao objetivo. O conclusão proposta é uma interpretação além dos resultados obtidos.

*A conclusão do trabalho foi reformulada, conforme proposto para refletir a resposta ao objetivo:*

*“In May-July 2020 the observed prevalence of anti-SARS-CoV-2 specific antibodies was low in a survey population and comparable to other studies developed in similar settings with low incidence rates of COVID-19. Given the low sensitivity of the National Surveillance System to detect asymptomatic cases we emphasize the need of monitoring the distribution of specific antibodies against SARS-CoV-2 during the forthcoming months, especially after the second pandemic wave, aiming to estimate the fraction of SARS-CoV-2 susceptible population and supporting vaccination strategy. ”*

**Revisor F**

**Title**

Study title is concise and clear on the main elements of the study, including an indication of study design. However, it has no time-bound reference concerning the study application.

*We reformulated the title as suggested, including reference to the study period (May-July 2020): “Seroprevalence of SARS-CoV-2 infection in Portugal in May-July 2020: results of the first National Serological survey (ISNCOVID-19)”*

**Abstract**

Well-structured and clear abstract providing an informative and balanced summary of what was done and what was found. Objectives are clearly defined. Study design is explicitly declared. General methods and statistical analysis are described, albeit without any mention of the study sampling process. Main findings are also clearly stated, including point estimates and confidence intervals. Initial claim stated on Discussion and Conclusions can be challenged by the generalizability of the results.

*We reformulated the methods section of the abstract and included reference to non-random method of participants selection. “Cross-sectional seroepidemiological study was developed after the peak of the first epidemic wave using a non-random sample of 2,301 Portuguese residents, aged 1 year or older.”*

*The conclusion section was also reformulated to answer the reviewer suggestion:*

*“Our results support limited extent of infection by SARS-CoV-2 in the study population possibly due to early lockdown measures implemented in Portugal support the need to continue monitoring of SARS-CoV-2 seroprevalence to increase our knowledge about epidemic´s evolution and to estimate the proportion of the susceptible population along time”.*

**Introduction**

* Scientific background is explained concisely and the study rationale is reported.

Study objective is clearly stated, albeit without any time bound reference.

*We corrected the objective as suggested. Time bound reference was added: “This study aimed to estimate the seroprevalence of SARS-CoV-2 specific antibodies (IgM and/or IgG) stratified by sex, age group, Health Region, education level and to determine the fraction of asymptomatic infections in May-July 2020”.*

**Methods**

* A reasonable description of study design is present early on in the methods section.

Study settings are described, mentioning overall setting, locations as well as dates for period of recruitment and data collection. There is no explicit mention on the eligible time period of retrospective assessment of exposure.

*Regarding the eligible time period of retrospective assessment of exposure we would like to clarify that participants were asked to report if they experienced any of signs and symptoms since March, the date when the first case of COVID-19 was officially detected in Portugal and its already mentioned in the text in the description of survey questionnaire.*

*“Data was collected through self-administrated survey questionnaire for sociodemographic, clinical and epidemiological information, including information on contact with a suspected or confirmed case of COVID-19 and presence of signs and symptoms compatible with SARS-CoV-2 infection since* ***March 202****0.”*

*At the time of study implementation we used this methodological option since it was recommended by the WHO protocol for seroepidemiological investigation protocol for COVID-19 virus infection. Currently, for further survey waves we consider that asking participants on signs and symptoms during explicit number of weeks before the interview would be a better option.*

- Eligibility criteria for participants are given clearly along with sources of participant recruitment. Methods for selection of participants and handling of non-participation could be added.

*Given that we used non-probability quota sampling, we instructed fieldwork teams to recruit any person who satisfy the eligibility criteria until the established quota by age group and sex for respective data collection point was completely filled. The fieldwork teams were instructed to check eligibility criteria and to report the number of individuals who were invited but were not eligible. Overall, 80 potential participants were considered not eligible. We did not instruct fieldwork staff to register refusals. For this reasons it is not possible for us to add an information on non-participants to the manuscript.*

- Sampling process is defined briefly. Sampling type is stated (non-probabilistic sampling) and parameters for sampling size calculation are given, albeit without a clear reference to the formula of calculation.

*Sample size was computed by age group, as based on attack rates reported by National Surveillance System we expected to observe smaller prevalence among minors. The following formula was used for sample size calculations in each age group:*

*where p stands for expected seroprevalence, N for population size, d for absolute precision (margin of error), DEFF for design effect and z is the 0.975 percentile of Standard Normal distribution. It is a standard formula to compute sample size to estimate proportion,* *a literature reference can be found in Schaeffer RL, Mendenhall W, Ott L. Elementary Survey Sampling, Fourth Edition. Duxbury Press, Belmont, California 1990.*

-The stratified sampling process is not explained clearly, in particular how municipalities within a given NUTS3 were selected and consequently how sampled participants were weighted. There is no mention of the sampling frame used for stratification. The whole process of sampling appears to be more complex than what has been described in the Methods. Figure 1 adds some information on the sampling strata but does not address the aforementioned issues.

*We agree with the reviewer comment on unclear sampling methods description. It was amplified the in the new version of the manuscript where we provide a more complete explanation of the methodological approach.*

*The following paragraphs were introduced:*

*““A two-stage non-probability sampling design (quota sampling) stratified by age group was used. In order to estimate an expected seroprevalence of 2.5% in the 0-19 yo and of 5% in the 20 yo and over, with an absolute precision of 2% for 95% confidence interval and a design effect of 1.5, the required sample size was determined to be 2,072 individuals.*

*Sampling units, individuals, were allocated homogeneously by Health Region (North, Centre, Lisbon and Tagus Valley, Alentejo, Algarve, and the AR of Madeira and Azores) in order to obtain estimates with similar precision in all regions. To assure survey geographical coverage in North, Centre, Lisbon and Tagus Valley, and Alentejo regions sample size was allocated, proportionally to the distribution of resident population, by NUT III, while in Algarve, Autonomous Region (AR) Madeira and AR Azores by smaller geographical units, since each of these three regions consist of a single NUT III.*

*Within each NUT III or equivalent smaller geographical unit, municipalities were selected by simple random sampling. The number of municipalities to select in each NUT III was proportional to geographical unit population size within Health Region (in larger NUT III more municipalities were selected). The final number of municipalities selected was 101.  Within each of randomly selected municipality, data collection point were chosen, by convenience, from a nationwide network of private and public pathology laboratories. At least one data collection point was selected in each regional unit (NUT III level). The number of data collection points was determined taking into consideration logistical issues, such as the number of days for observation and capacity to recruit participants (17-22 individuals in each data collection point) after lockdown restrictions were lifted. The selected laboratories consisted of 96 clinical pathology laboratories and 18 public hospitals located in 101 randomly selected municipalities (Fig 1). Survey participants were recruited according pre-defined sex–age group quota distributed by Health Region (homogenously), by NUT III (proportional to population size), by selected municipality (homogeneously within NUT III).”*

*In fact, our sampling scheme was more complex. The final step, the sampling of individuals, was non-random by quota sampling. However, the selection of municipalities to perform data collection was done by simple random sampling in each NUT III. We considered very important to have a good geographical coverage, and, at least, select randomly data collection places since random selection of participants was not feasible given logistical and mainly time restrictions.*

*Our sample was stratified by age group, this methodological option was chosen because of evidence of different attack rates reported by National Surveillance System. Based on surveillance data we expected to observe lower seroprevalence in younger age groups.*

*When defined quotas we allocated sample by sex proportionally to the distribution of resident population.*

*We decided on allocate participants homogeneously by region in order to obtain estimates with similar precision is each of health regions, so to inform regional public health authorities.*

*Given the fact that number of participants required by age group to estimate a prevalence was not proportional to the distribution of the Portuguese population and homogeneous allocation of the sample by region we have to use of sampling weight that corrects sample structure to compute prevalence estimates. To compute sampling weight we used methodology identical to previously applied by Inquerito Serológico Nacional a Doenças Evitaveis por Vacinação, developed in 2015-16. We added respective a reference in the text to explain sampling weight calculations.*

- Variables regarding outcome and exposures are briefly mentioned. Elements pertaining to the survey questionnaire could be described more thoroughly. Diagnostic criteria for outcome assessment are given clearly alongside case definitions. A substantial part of exposure assessment was done by survey questionnaire collecting self-reported exposure. The method of application of the questionnaire is not stated nor described in detail (e.g. Self-administered? Interviewer-administered? Computer-assisted personal interviewing (CAPI)? Computer-Assisted Self Interviewing (CASI)?). The time window for exposure assessment on the survey questionnaire is not stated.

*We added data collection approach as required, the time window for exposure assessment on the survey questionnaire was also reported. “The fieldwork took place between May 21 and July 8, 2020. Data was collected by in paper self-administrated survey questionnaire…..”*

-Concerning bias analysis, there is no clear description to address potential sources of bias, namely selection bias due to the sampling process and collider bias due to the context of clinical pathology laboratory sample catchment and both laboratory and hospital referral. Potential bias are mentioned in the discussion section.

*We added more profound discussion of bias in the discussion section.*

*“Among limitations that could have affected the study results, we should mention the lack of representative sample of general Portuguese population because research has shown that surveys on SARS-CoV-2 seroprevalence may be seriously affected by self-selection or volunteer bias17 as individuals with previous diagnosis of COVID-19 volunteered more frequently to participat 17. To reduce this self-selection bias in the ISNCOVID-19 survey participants were recruited among those who already were in a laboratory with the objective to perform routine blood tests prescribed by a medical doctor for reasons unrelated to COVID-19. This methodological option, on the other hand, may have resulted in sampling of individuals with higher proportion of chronic conditions than in general population18, particularly among children, and this could have contributed to a lower seroprevalence resulting from a higher awareness and adoption of protection measures by these groups at risk. In addition, selecting participants among users of clinical pathology laboratories may have resulted in bias due to the documented educational gradient in access to complementary diagnostic tests18. However, the Portuguese Serological Survey for Vaccine Preventable Disease developed in 2015-2016 using similar sampling strategy among clinical pathologies laboratories and public hospital users did not demonstrate bias in the educational distribution of participants5. Exclusion of institutionalized individuals from the present survey may have contributed to underestimation of SARS-CoV-2 seroprevalence since, according to the national surveillance system, several institutional facilities for the elderly were affected by COVID-19 during the first wave.”.*

-Statistical methods mention a weighting process of estimates. However, it is unclear how that weighting is performed. Is a weight applied to each participant according to his sampling unit weight or does it refer to a post-stratification weighting using the general population as reference strata?

*To correct sample structure for design features (allocation by Health Region and age group), for each participant in age group , sex and region , sampling weight () was computed as following:*

*where stands for overall population size, for overall sample size, for sample size in age group , sex and region e for population size in age group , sex and region . Most recent available population figures provided by National Institute of Statistics were used. To compute prevalence estimates Stata [svy] routines were used.*

- There is no clear explanation on how variables were handled in the analyses and particularly how groupings were chosen and why.

*We added the following clarification to the manuscript to explain our choices. “Survey questions were selected based on WHO recommendations for SARS-COV-2 seroepidemiological studies2.”*

- There is no description on how missing data was handled.

*We clarified a treatment of missing data adding the following sentence to the statistical analysis section.” No imputations were performed, cases with missing data were excluded from analysis.”*

- Software for statistical analysis and specific packages for particular operations should be clearly reported including their version.

*We added a software description as required. “The data was analyzed using Stata 15.1® software4.”*

**Results**

- Numbers of individuals at each stage of study, namely those potentially eligible, examined for eligibility, confirmed eligible, included in the study, and analysed are not reported. There is no description of non-participants and no participant flowchart.

*As a non-random sampling was used, fieldwork staff registered only those who were invited and screened for eligibility. Overall, 80 invited individuals were not eligible due to disease/condition that affects an immune response. The refusals were not registered. For this reason we were not able to present flowchart and reported in the manuscripts only participants who were excluded from the analysis due to missing data on eligibility criteria or incomplete informed consent.*

- Characteristics of study participants are given in Table 1. This table is well structured and independent of the main body of text for its interpretation. There is no clear indication of the number of participants with missing data for each variable of interest.

*To improve Table 1 we added a number of cases analyzed for each variable.*

- Variables education level, previous contact with suspected/confirmed COVIS-19 case and self-reported symptoms have missing data that is not clearly stated (the sum of the absolute counts does not equal 2,301).

*Education level, due to Ethics Commission requirement, was only analyzed for adult participants, aged 20+ . That’s why the sum of counts is not equal to 2301. To clarify this point we added a footnote to the Table 1.*

-A characterisation on laboratory sample provision is missing (clinical laboratory and hospital laboratory).

*We added number of participants recruited by hospitals and by laboratories in the text. “Overall 2,301 participants were recruited, 1,467 by clinical pathology laboratories network and 834 by public hospitals.”*

-Table 2 displays the main findings for outcome data, including summary and stratified results, and weight-adjusted point estimates and their 95% confidence interval. This table is well structured and independent of the main body of text for its interpretation, with proper column and row headings, appropriate numerical representation with two significant digits for estimates and p-values and correct footnotes. Nevertheless, it would be interesting to have a crude estimate displayed.

*Given the sample design it does not seem methodologically correct to us to produce unweighted estimates. For this reason we will not include any changes in the table 2.*

-Results state that a given seroprevalence estimate was observed at the national level. Due to the issues with non-probabilistic sampling and statistical inference to the general population, this might be readdressed.

*We reformulated the sentence, replacing “at national level” by “overall” to reflect more accurately that survey inference based on non-random sampling.*

**Discussion**

Firstly, it provides a summary of key results that focus on the study objective.

Secondly, it frames the main findings with concurrent literature on the topic, providing some explanation for the observed results.

There is a brief mention on the limitations from potential recall bias and non-random sample bias, even though without any discussion on both direction and magnitude of these potential biases.

Constraints on the generalizability of the study results are mentioned and acknowledged, although without suggesting a possible alternative for future implementation or a tentative analytical procedure to curtail part of this limitation. Conclusion in the main body of text is discrepant in comparison with the one in the abstract.

*We expanded the discussion on the study limitations. “Among limitations that could have affected the study results, we should mention the lack of representative sample of general Portuguese population because research has shown that surveys on SARS-CoV-2 seroprevalence may be seriously affected by self-selection or volunteer bias17 as individuals with previous diagnosis of COVID-19 volunteered more frequently to participat 17. To reduce this self-selection bias in the ISNCOVID-19 survey participants were recruited among those who already were in a laboratory with the objective to perform routine blood tests prescribed by a medical doctor for reasons unrelated to COVID-19. This methodological option, on the other hand, may have resulted in sampling of individuals with higher proportion of chronic conditions than in general population18, particularly among children, and this could have contributed to a lower seroprevalence resulting from a higher awareness and adoption of protection measures by these groups at risk. In addition, selecting participants among users of clinical pathology laboratories may have resulted in bias due to the documented educational gradient in access to complementary diagnostic tests18. However, the Portuguese Serological Survey for Vaccine Preventable Disease developed in 2015-2016 using similar sampling strategy among clinical pathologies laboratories and public hospital users did not demonstrate bias in the educational distribution of participants5. Exclusion of institutionalized individuals from the present survey may have contributed to underestimation of SARS-CoV-2 seroprevalence since, according to the national surveillance system, several institutional facilities for the elderly were affected by COVID-19 during the first wave.”.*

*We reformulated the study conclusion to reflect the actual results.*

*“In May-July 2020 the observed prevalence of anti-SARS-CoV-2 specific antibodies was low in a survey population and comparable to other studies developed in similar settings with low incidence rates of COVID-19. Given the low sensitivity of the National Surveillance System to detect asymptomatic cases we emphasize the need of monitoring the distribution of specific antibodies against SARS-CoV-2 during the forthcoming months, especially after the second pandemic wave, aiming to estimate the fraction of SARS-CoV-2 susceptible population and supporting vaccination strategy. ”*

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